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Metabolic Reprogramming Is Required for Antibody Production That Is Suppressed in Anergic but Exaggerated in Chronically BAFF-Exposed B Cells

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B cell activation leads to proliferation and Ab production that can protect from pathogens or promote autoimmunity. Regulation of cell metabolism is essential to support the demands of lymphocyte growth and effector function and may regulate tolerance. In this study, we tested the regulation and role of glucose uptake and metabolism in the proliferation and Ab production of control, anergic, and autoimmune-prone B cells. Control B cells had a balanced increase in lactate production and oxygen consumption following activation, with proportionally increased glucose transporter Glut1 expression and mitochondrial mass upon either LPS or BCR stimulation. This contrasted with metabolic reprogramming of T cells, which had lower glycolytic flux when resting but disproportionately increased this pathway upon activation. Importantly, tolerance greatly affected B cell metabolic reprogramming. Anergic B cells remained metabolically quiescent, with only a modest increase in glycolysis and oxygen consumption with LPS stimulation. B cells chronically stimulated with elevated BAFF, however, rapidly increased glycolysis and Ab production upon stimulation. Induction of glycolysis was critical for Ab production, as glycolytic inhibition with the pyruvate dehydrogenase kinase inhibitor dichloroacetate sharply suppressed B cell proliferation and Ab secretion in vitro and in vivo. Furthermore, B cell–specific deletion of Glut1 led to reduced B cell numbers and impaired Ab production in vivo. Together, these data show that activated B cells require Glut1-dependent metabolic reprogramming to support proliferation and Ab production that is distinct from T cells and that this glycolytic reprogramming is regulated in tolerance. The Journal of Immunology, 2014, 192: 000–000.

Proper regulation of lymphocyte function is critical to allow normal immune responses while preventing autoimmunity or immunodeficiency. Lymphocyte metabolism is now appreciated to play a key role in cellular function and homeostasis (1). In T lymphocytes, activation through the TCR along with CD28-mediated costimulation leads to a rapid increase in expression of the glucose transporter, Glut1, to support increased glucose uptake and metabolism (2, 3). Simultaneously, glutamine oxidation increases and β-oxidation of fatty acids decreases (4, 5). Overall, glycolysis becomes predominant over oxidative metabolism in activated T cells, leading to a reliance on aerobic glycolysis and glutaminolysis in a metabolic phenotype that resembles that of cancer cells (4, 6). Stimulated dendritic cells and inflammatory macrophages induced similar metabolic programs (7, 8). Metabolic reprogramming in T cells is mediated through the induction of cMyc (5), a well-described regulator of glycolysis, glutaminolysis, and cell growth, together with the PI3K/Akt pathway (2). As in T cells, the PI3K/Akt pathway can increase B cell expression of Glut1 and metabolism upon antigenic stimulation (9, 10). There can be significant metabolic heterogeneity in distinct lymphocyte subsets (11), and it is now of significant interest to establish mechanisms of metabolic reprogramming to better understand lymphocyte physiology and identify metabolic targets that could be exploited to treat disease. However, the metabolic phenotype of stimulated B cells and the requirements for Ab production are poorly understood.

Metabolic reprogramming to induce glycolysis may dictate the inflammatory potential of activated lymphocytes. Glucose deprivation (3, 12, 13), or treatment with the glycolytic inhibitor, 2-deoxyglucose (2-DG) (5, 14, 15), suppresses T cell activation, proliferation, and production of IFN-γ. In contrast, increased glucose uptake can enhance T cell function. Transgenic expression of Glut1 increased glucose uptake and metabolism in T cells and led to a larger basal cell size and hyperactivation of transgenic T cells with elevated IL-2 and IFN-γ production and more rapid proliferation when stimulated (3). Over time, T cell–specific Glut1 transgenic animals developed lymphadenopathy and splenomegaly, with hyper–γ-globulinemia and glomerular immune complex deposition.

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deposition at 1 y of age (3, 16), demonstrating increased glucose metabolism can enhance lymphocyte function.

Metabolic reprogramming to support lymphocyte activation, however, is not uniform, and distinct stimuli promote metabolic pathways to match the needs of specific cell functions. Depending on the cytokine environment, activated CD4 T cells differentiate into inflammatory effectors, such as Th1 and Th17 cells, or immunologic suppressors, T regulatory cells (17). Th1 and Th17 CD4 T cells express high levels of Glut1 and depend on glycolytic flux (16, 18, 19). T regulatory cells, however, have lower levels of Glut1 expression and instead rely on mitochondrial metabolism and lipid oxidation (16, 19). Macrophage M1 and M2 subsets follow a similar pattern, with inflammatory M1 macrophages being predominantly glycolytic, whereas anti-inflammatory M2 macrophages use lipid oxidation (7, 20). Metabolic reprogramming of activated effector T cells to favor glycolysis and lactate production is then reversed back to an oxidative phenotype at the conclusion of an immune response, with memory CD8 lymphocytes decreasing glycolysis and instead relying on lipid oxidation (21, 22). Similar to the reduced glycolysis of anergic (23) or memory T cells (22), B cell activation can fail to induce glycolysis if FcγRIIB is coligated (9). Tolerance-inducing and immunosuppressive mechanisms can, therefore, prevent or modify metabolic reprogramming.

B cell tolerance mechanisms are well defined and include apoptosis, receptor editing, and the induction of anergy (24). However, these tolerance mechanisms can be prevented or overridden by chronic cytokine stimulation to promote autoimmunity, such as can occur in systemic lupus erythematosus (SLE). For example, increased levels of the cytokine BAFF are associated with SLE (25, 26), and transgenic BAFF overexpression to chronically expose B cells to elevated levels of BAFF leads to a spontaneous SLE-like disease in mice (27, 28). Importantly, BAFF can activate the PI3K/Akt signaling pathway (29) and promote glucose utilization in B cells (30). BAFF inhibition is a promising new biologic therapy in SLE (25), yet the impact of chronic BAFF exposure on B cell metabolism and roles of altered cellular metabolism in autoimmunity are uncertain.

Lymphocyte metabolism may provide a new opportunity to modulate immunity and inflammatory disease. In this study, we examine the regulation of B cell metabolism upon activation and the metabolic effects of anergy or chronic BAFF stimulation and autoimmunity. Surprisingly, we show that B cells are metabolically distinct from T cells and do not switch to predominantly favor glycolysis but instead increase metabolism in a balanced fashion. Anergy and chronic BAFF overexposure led to broad and opposing changes in B cell metabolic capacity, with anergy-suppressing and chronic BAFF overexposure enhancing cell metabolism. In particular, B cells from BAFF transgenic mice were primed to rapidly increase glycolysis upon stimulation. These changes were critical for B cell function, as inhibition of glycolysis or B cell–specific deletion of Glut1 suppressed Ab production in vivo. Therefore, B cells rely on Glut1, and targeting B cell metabolic regulation and glycolytic pathways may provide a new tool to prevent B cell proliferation and autoimmune body production.

Materials and Methods

**Mice**

C57BL/6, RAG1<sup>−/−</sup>, Hif1α<sup>−/−</sup>, MD4 ML5, and CD19-Cre transgenic mice were obtained from The Jackson Laboratory. BAFF transgenic mice that express full-length BAFF driven by the myeloid cell–specific CD68 promoter (founder MB21) were provided by D. Nemazee (Scirpts Research Institute) (31). Myc<sup>+/−</sup> mice (provided by F. Alt, Harvard) (32) were backcrossed six generations onto the C57BL/6 background. Both Myc<sup>−/−</sup> and Hif1α<sup>−/−</sup> mice were crossed with ROSA26CreER<sup>22</sup> (33). Glut1<sup>−/−</sup> mice (34) were crossed to CD19-Cre transgenics. The acute deletion of Myc or hypoxia-inducible factor-1 (HIF1)α was achieved through in vivo delivery of tamoxifen (1 mg/mouse, i.p.) 3 d before B cell isolation. Some animals were treated with dichloroacetate (DCA; 2 g/L in drinking water changed twice each week). For bone marrow reconstitution, RAG1<sup>−/−</sup> mice were lethally irradiated with two doses of 4.5 Gy and provided wild-type bone marrow by tail vein injection. Sex-matched 7- to 12-wk-old mice were used throughout. Mice were housed and cared for at Duke University or St. Lucie Children’s Research Institute (Duke University). Use Committee-approved protocols. Human B cells were isolated from healthy donor peripheral blood (Gulf Coast Regional Blood Center).

**Cell isolation and reagents**

Splenic naive B or T cells or human peripheral blood B cells were isolated by magnetic bead negative selection (purity was typically >90%; Milteny Biotec) and cultured in RPMI 1640 (Mediatech) supplemented with 10% FBS, 2% x-Gene Bio-Products, HEPES, and 2-ME. B cells were stimulated with 10 μg/mL LPS (Sigma, Aldrich), 20 μg/mL F(ab)′<sub>2</sub>; anti-IgM (Jackson ImmunoResearch Laboratories), or CpG oligodeoxynucleotide (ODN) (InvivoGen; catalogue ODN-2006). T cells were treated in plates coated with 10 μg/mL CD3 and CD28 (eBioscience). Unstimulated B cells were maintained in 20 ng/mL BAFF (R&D Systems) to maintain in vitro viability. Some cultures were treated as indicated with 2-DG (0.5 mM; Sigma-Aldrich), 10 μM DCA (VWR), or low-dose rotenone (80 nM; Seahorse Bioscience).

**Flow cytometric analysis and Abs**

Cytometry analysis was performed with a MACSQuant Analyzer (Miltenyi Biotec) and analyzed with FlowJo software (Tree Star). Anti-mouse CD19 allophycocyanin, CD69 PE, IgM FITC, and IgD Vioblu (eBioscience) or anti-human CD69 FITC (Miltenyi Biotec) were used to measure purity and B cell activation. Cells were incubated 30 min with 200 nM Mitotracker Green (Invitrogen) and washed to measure mitochondrial content. Proliferation was analyzed by CFSE staining and flow cytometric measurement of CFSE dilution. Glut1 expression was measured by intracellular flow cytometry of fixed cells using monoclonal anti-Glut1 (Abcam; Ab652) in the presence of rat serum and Fc block, followed by anti-rabbit PE before flow analysis.

**Quantitative RT-PCR**

RNA was harvested from purified B cells (RNeasy Plus; Qiagen) ex vivo or following stimulation with anti-IgM or LPS and reverse transcribed (iScript; Bio-Rad) to perform SYBR Green–based (Bio-Rad) quantitative RT-PCR of Glut1 (forward, 5′-AGCCCTGTGCTAGTGATTGTA′<sub>3</sub>−3; reverse, 5′-AGG TCTCGGGTGTACACATC′<sub>3</sub>−3′) and cMyec (forward, 5′-CTGTGTGGAAGGCT-<sub>3</sub>′ GGATTTCTC′<sub>3</sub>−3′; reverse, 5′-CAGCACCAGACAGCAGC′<sub>3</sub>−3′). Results were normalized to B-2-microglobulin (forward, 5′-GAG AAT GGG AAG CGG AAC ATA′<sub>3</sub>′; reverse, 5′-GCTGAAAGGACATCTGCTGAC′<sub>3</sub>−3′).

**Western blot**

Cells were lysed in a low detergent buffer (1% Triton, 0.1% SDS) for 1 h with protease and phosphatase inhibitors (Sigma-Aldrich). Nicotinellol cellulose membranes were hybridized with anti-phospho S232/233-PADE-1Xα (Millipore; AP1063), total PDH-E1α Abs (Abcam; ab110334), Glut1 rabbit monoclonal (Abcam; ab115730), Glut3 rabbit polyclonal (Abcam; ab15311), actin (Cell Signaling; 4970S), cMyec (Cell Signaling; 179), or Hif1α (Cayman Chemicals; 10006421).

**Metabolic assays**

Glucose uptake (35), glycolytic flux, hexokinase activity, fatty acid β-oxidation, glucose oxidation, glutamine oxidation, and pyruvate oxidation were measured, as previously described (5). Briefly, glucose uptake was measured by incorporation of 2-deoxy-[<sup>3</sup>H]glucose. Glycolytic flux was determined by measuring the detritiation of [3H]-glucose. Glucose, glutamine, and pyruvate oxidation were measured by culture of cells in U-13C glucose, glutamine, and pyruvate, respectively, to measure production of [13C]<sub>2</sub>CO<sub>2</sub>. [3H]-Palmitic acid was used to measure lipid oxidation by the production of [3H]<sub>2</sub>H<sub>2</sub>O. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured with a XF24 extracellular flux analyzer (Seahorse Biosciences). Briefly, 1.4 × 10<sup>6</sup> unstimulated or 10<sup>6</sup> stimulated cells/well were seeded in a Cell-Tak (BD Biosciences)–coated plate, and OCR and ECAR measurements were normalized to cell number. Cells were initially plated in XF Seahorse media with glutamine alone when glucose was injected in ECAR tests, or both glucose and glutamine in mitochondrial stress test using the following concentrations of injected compounds, as indicated in the text: oligomycin, 1 μM; rotenone,
glycolytic capacity was calculated after addition of oligomycin. (B) Basal OCR was determined after addition of glucose (10 mM), oligomycin (1 \( \mu \)M), and 2-DG (20 mM), as indicated. (B) Basal OCR was determined after addition of glucose, and glycolytic capacity was calculated after addition of oligomycin. (C and D) Purified B cells were cultured 24 h in BAFF (20 ng/ml) to maintain viability (unstimulated [UNS]) or stimulated with LPS, and (C) glycolytic rate and (D) glucose oxidation were directly calculated using radiolabeled glucose. (E and F) Purified B cells were examined after 6 h culture without stimulation (UNS) or with LPS, and OCR was determined by extracellular flux analysis. (E) Representative plot of OCR over time with addition of oligomycin (1 \( \mu \)M), mitochondrial uncoupler FCCP (0.5 \( \mu \)M), and electron transport inhibitors antimycin (1.5 \( \mu \)M) + rotenone (0.75 \( \mu \)M), as indicated. (F) Basal OCR was determined prior to addition of oligomycin, and maximal respiratory capacity was determined by subtracting nonmitochondrial OCR, calculated after antimycin and rotenone injection to maximal OCR upon FCCP uncoupling and maximal electron transport. (G) The ratio of basal OCR/ECAR was determined in purified unstimulated and LPS-treated B cells. Values from 24-h stimulation are normalized to unstimulated cells. (A, B, C, and E are representative data from one of three independent experiments. (C and D) show means from three or more independent experiments. Means and SDs are shown, and statistical significance was determined by Student’s \( t \) test (*\( p \leq 0.05 \), **\( p < 0.005 \), N.S., not significant).
ex vivo or Ag receptor stimulated for 24 h, and several key metabolic differences were observed. First, unstimulated B cells had similar oxygen consumption (OCR; Fig. 2A) but significantly higher rates of glycolysis (ECAR; Fig. 2B) than T cells. Second, whereas B cells increased both OCR and ECAR equivalently after activation, the extent of increase was lower than that observed in T cells. Lastly, Ag-stimulated B cells maintained a balanced OCR/ECAR ratio, whereas activated T cells shifted to a glycolytic metabolism (Fig. 2C). Thus, B cell metabolism is reprogrammed after LPS or Ag receptor stimulation, but B cell metabolism differs from resting or stimulated T cells.

Glut1 and mitochondrial content

B cell metabolic reprogramming was balanced and involved proportionally increased expression of both glycolytic and mitochondrial components. One essential pathway to increase glycolysis and glucose metabolism is through increased glucose uptake. Indeed, stimulation of murine B cells through anti-IgM or LPS or human peripheral blood B cells with anti-IgM for 24 h each significantly increased glucose transport when measured by uptake of radiolabeled glucose (Fig. 3A). Glucose uptake is mediated through a family of facilitative glucose transporters that are differentially expressed in cells of distinct lineages and differentiation states. Of these transporters, Glut1 is highly expressed in hematopoietic cells and B cell activation increased Glut1 mRNA (Fig. 3B) and protein (Fig. 3C). In addition, B cell activation led to a rapid overall increase in mitochondrial mass, as evidenced by increased staining with Mitotracker Green within 6 h of stimulation (Fig. 3D). Even with distinct stimulatory signals through TLR4 or the BCR, therefore, B cells initiated similar metabolic reprogramming events that affect both glucose uptake and mitochondria.

B cell metabolic reprogramming is HIF1α independent yet requires cMyc

HIF1α and cMyc can induce transcription of metabolic genes involved in cell proliferation, including Glut1 (38). cMyc also plays a key role to promote glutaminolysis and mitochondrial biogenesis that may be important in lymphocyte activation and proliferation. T cell activation has been shown to require cMyc, but not HIF1α, to induce aerobic glycolysis in initial activation (5). Conversely, Th17 T cells require HIF1α (18, 19). We therefore tested the roles of HIF1α and cMyc in B cell metabolic reprogramming following activation. Despite efficient deletion of HIF1α (Supplemental Fig. 1B), wild-type and HIF1α-deficient B cells increased glycolysis equivalently after LPS stimulation (Fig. 4A). Therefore, like T cells (5), metabolic reprogramming in B cell activation does not require HIF1α. B cell activation through anti-IgM or LPS also led to rapid cMyc induction prior to cell cycle entry (Fig. 4B). In contrast to HIF1α and similar to T cells (5), cMyc was essential for activation-induced B cell upregulation of Glut1 (Fig. 4C) and glycolysis (Fig. 4D). Myc-dependent metabolic reprogramming was also evident by extracellular flux analysis, as Myc-deficient B cells failed to increase extracellular acidification rate that reflects glycolytic lactate production (ECAR; Fig. 4E) and mitochondrial oxygen consumption (OCR; Fig. 4F). Glutamine oxidation also increased in B cell activation and was Myc dependent (Fig. 4G). Similar to T cells (5), however, not all metabolic pathways of activated B cells were Myc dependent. B cell stimulation reduced lipid oxidation (Fig. 4H) and increased pyruvate oxidation (Fig. 4I) regardless of cMyc expression. Although additional regulatory pathways also contribute, these data show that cMyc plays a key role in the initial metabolic reprogramming of stimulated B cells.

Anergic B cells are metabolically suppressed

Chronic exposure of immature or transitional B cells with self-Ag can lead to the self-tolerance mechanism of anergy, in which lymphocytes become desensitized to stimulation for proliferation and Ab production (39, 40). To what extent desensitized signal transduction events prevent metabolic reprogramming, however, has not been established. The metabolic response of anergic B cells was analyzed in response to LPS and Ag receptor stimulation with anti-IgM. B cells from anti–hen egg lysozyme (MD4) Ig transgenic mice crossed to soluble hen egg lysozyme (ML5) transgenic mice are chronically exposed to self-Ag and rendered anergic (41). Control and MD4 ML5 transgenic B cells were stimulated for 6 h with anti-IgM and analyzed by extracellular flux analysis for ECAR and OCR (Fig. 5A). Control B cells responded to anti-IgM with increased OCR and ECAR basal and maximal capacity. Anti-IgM–stimulated MD4 ML5 transgenic B cells, in contrast, did not increase ECAR or OCR and maintained a metabolic phenotype similar to unstimulated B cells. Six-hour LPS stimulation of anergic MD4 ML5 B cells was sufficient to increase metabolism relative to resting control B cells (Fig. 5B). This increase in basal and maximal OCR and ECAR, however, was only partial, and anergic B cells remained less metabolically active than LPS-stimulated control MD4 B cells.

B cells chronically exposed to high levels of BAFF have increased metabolic capacity

Consistent with association with SLE (25–28, 31), B cells from the MB21 line of BAFF transgenic mice have elevated Ab production.
B cell stimulation increases both Glut1 and mitochondrial mass. (A–C) Purified B cells were cultured without stimulation, with anti-IgM, or with LPS, and (A) glucose uptake in purified mouse or human B cells (left and right panels, respectively) was measured using radiolabeled glucose; (B) Glut1 mRNA in murine B cells was measured by quantitative RT-PCR; and (C) Glut1 protein levels in murine B cells were determined by intracellular flow cytometry. (D) Purified murine B cells were cultured without stimulation or with LPS for 6 h and stained with Mitotracker Green to measure mitochondrial mass by flow cytometry. Representative data from three or more experiments are shown for (A), (C), and (D). The average of three independent experiments is shown in (B). Means and SDs are shown, and statistical significance was determined by Student’s t test (**p < 0.005).

The broad upregulation of glucose and mitochondrial metabolic pathways upon B cell activation suggested that B cells have potential metabolic flexibility to withstand loss of specific nutrients. To test whether B cells rely on glucose metabolism, isolated B cells were stimulated in the presence of 2-DG or pyruvate dehydrogenase kinase inhibitor DCA. These inhibitors impair glucose metabolism at distinct steps, as 2-DG prevents glucose entry into glycolysis, whereas DCA blocks PDHK-mediated phosphorylation of pyruvate dehydrogenase (Supplemental Fig. 3A) and promotes pyruvate entry into the tricarboxylic acid cycle rather than conversion to lactate. Although acting at the proximal and distal steps of glycolysis, both 2DG and DCA reduced ECAR (Figs. 1A, 4A, 4B, 5A, Supplemental Fig. 3B). Treatment with a low dose of 2-DG strongly suppressed the proliferation of LPS-stimulated B cells (Fig. 7A). Importantly, LPS-induced secretion of IgG and IgM was curtailed with low-dose 2-DG (Fig. 7B, 7C). Similarly, DCA was nontoxic (Supplemental Fig. 3C) and, despite normal induction of B cell early activation markers (Supplemental Fig. 3D), sharply suppressed B cell proliferation and Ab production (Fig. 7A, 7B). DCA also suppressed proliferation and Ab secretion from human B cells stimulated with the TLR9 ligand, ODN (Fig. 7D, 7E). Because oxygen consumption increased in activated B cells, the dependence of IgM production on mitochondrial electron transport was also tested by treatment with rotenone. Consistent with a primary dependence on glycolysis, rotenone had no effect on IgM secretion (Fig. 7C). Therefore, despite the broad increase in metabolism of activated B cells, glycolysis and the specific conversion of pyruvate to lactate rather than acetyl-CoA appear essential for proliferation and Ab secretion.

Inhibition of pyruvate dehydrogenase kinase suppresses in vivo Ab production

We next tested the dependence of B cells on glycolytic flux for Ab production in vivo. To examine homeostatic Ab production, RAG1-deficient mice that lack endogenous Abs were lethally irradiated and reconstituted with wild-type bone marrow. Mice were then provided control water or drinking water containing DCA to suppress aerobic glycolysis and instead promote glucose oxidation. DCA treatment did not affect lymphoid reconstitution, as numbers of both peripheral blood B and T cells were unchanged with DCA treatment (Fig. 7F). Importantly, recovery of total serum IgG was sharply
suppressed with DCA treatment after 20 d of reconstitution (Fig. 7G). After 70 d, however, IgG levels in DCA-treated mice reached normal levels. The in vivo dependence of Ab production on high rates of glycolytic flux following acute stimulation was next examined by immunization in the presence of DCA. Mice were immunized with NP-OVA and treated with normal or DCA-containing drinking water, and serum Ab levels were assessed after 15 and 19 d (Fig. 7H). Importantly, production of NP-reactive anti-NIP Ab (36) was suppressed by DCA treatment.

Glut1 is essential for B cell homeostasis and Ab production
Pharmacologic inhibition of glycolysis suppressed B cell proliferation and Ab production, but the cell-intrinsic dependence of B cells on glycolysis in vivo was unclear. To directly test the role of glucose uptake and glycolysis in B cell function, Glut1fl/fl mice were crossed to CD19-Cre transgenics to specifically delete Glut1 in B cells. Glut1 deficiency lowered peripheral B cell numbers, with a specific reduction in the number of IgMbrightB220+ mature B cells (Fig. 8A, 8B) to demonstrate a role for Glut1 in B cells. An increased percentage of IgMlowB220+ cells in Glut1fl/fl CD19-Cre mice was also IgD+, suggesting increased frequency of immature B cells or possibly a population of B cells that had undergone class switch. These peripheral B cells had, however, deleted Glut1, and Glut1fl/+ CD19-Cre did not express Glut1, whereas control B cells expressed and upregulated Glut1 upon activation with LPS or anti-IgM (Fig. 8C). There was no apparent compensation through the related glucose transporter, Glut3, as control and Glut1-deficient resting B cells equivalently expressed and downregulated Glut3 after activation. Importantly, total serum IgM levels were significantly reduced in Glut1fl/fl CD19-Cre mice (Fig. 8D). At 7 days after immunization with NP-OVA, anti-NIP-reactive NIP5 (high-affinity) and NIP25 (high- and low-affinity) IgM and IgG were increased in control mice. Glut1fl/fl CD19-Cre mice, however, failed to efficiently induce total or NP-specific IgM of IgG secretion. Total IgM and IgG serum levels were also significantly lower in Glut1fl/fl CD19-Cre mice. Specific Ab production also remained low 15 and 21 d after immunization, and the differences in total Ab levels persisted (Supplemental Fig. 4). Together, these data show that B cells require Glut1 and glucose uptake to maintain B cell populations and to support metabolic reprogramming necessary for maximal Ab secretion in vivo.

Discussion
Lymphocyte metabolic needs must be met to sustain cell viability and to allow cell functions, including proliferation and effector activity (1). T cell activation has been shown to increase glucose metabolism as part of the adaptive immune response, which is crucial for antigen-driven proliferation and differentiation of T cells. The role of glucose metabolism in B cells, particularly in the context of Ab production, has been less well characterized. Glut1 is essential for B cell metabolism and Ab production, as its deficiency leads to reduced B cell numbers and impaired Ab secretion.

Glucose is a key substrate for B cells, and its uptake is necessary for the expression of certain metabolic enzymes and pathways. Pharmacologic inhibition of glycolysis with 2-deoxy-D-glucose (2-DG) or DCA induces EAE, and Glut1 is essential for EAE induction (27). These findings suggest that Glut1 may play a crucial role in B cell metabolism and function.

In summary, Glut1 is essential for B cell homeostasis and Ab production. Glucose uptake and glycolysis are critical for B cell function, and the metabolic requirements of B cells are met to support Ab production. The role of Glut1 in B cell metabolism and Ab production highlights the importance of glucose metabolism in immune cell function and suggests potential targets for therapeutic intervention in autoimmune diseases.
uptake and glycolysis, leading to a predominantly glycolytic phenotype that is also characteristic of many cancer cells. B cells have also been shown to increase glucose metabolism upon stimulation (9, 10, 14). The details and relevance of this B cell metabolic transition from quiescence to activation, however, are not understood. In this study, we examined the glycolytic and mitochondrial metabolism of resting and stimulated normal, anergic, or chronically BAFF-stimulated and autoimmune prone

FIGURE 5. Anergic B cells are metabolically suppressed, whereas BAFF transgenic B cells are primed for metabolic reprogramming. (A and B) Purified B cells from control (wild-type [WT]), MD4 transgenic, or MD4 ML5 double-transgenic mice were cultured without stimulation or were stimulated with (A) anti-IgM or (B) LPS for 6 h. Representative plots are (left) of B cell OCR over time with addition of oligomycin (1 μM), mitochondrial uncoupler FCCP (0.5 μM), and electron transport inhibitors antimycin A (1.5 μM)+ rotenone (0.75 μM), as indicated. Representative plots are (right) of B cell ECAR over time with addition of glucose (10 mM), oligomycin (1 μM), and 2DG (20 mM), as indicated. (C and D) Purified B cells from control (WT) or BAFF-transgenic mice were cultured without stimulation (UNS) or with LPS for 6 h. Metabolic inhibitors were added during the assays, as indicated. (C) Representative plots of B cell OCR (left) and ECAR (right) are shown. (D) Basal and maximal OCR and ECAR from B cells from control and BAFF-transgenic mice stimulated with LPS for 6 h. (E) Glucose uptake of B cells cultured without stimulation or with LPS for 24 h measured using radiolabeled glucose. Means and SDs are shown, and statistical significance was determined by Student’s t test (*p ≤ 0.05, **p < 0.005. N.S., not significant). Data are representative of three or more experiments.

FIGURE 6. Chronic BAFF overexposure leads to a glycolytic shift upon activation not observed in normal or tolerant B cells. Purified B cells from wild-type control (WT), BAFF transgenic, and MD4 ML5 transgenic mice were LPS stimulated, and OCR and ECAR were measured. The ratios of OCR/ECAR were normalized to unstimulated B cells (UNS) after 6, 12, and 24 h. Data shown are means from three or more independent experiments, and statistical significance was determined by Student’s t test (**p ≤ 0.005. N.S., not significant).
B cells. Activation of normal B cells led to broadly increased metabolism but decreased lipid oxidation. Interestingly, unlike T cells or macrophages, normal B cells do not readily transition to a glycolytic metabolism and instead increase metabolism without specific shifts in the balance of lactate production to oxygen consumption. Anergic B cells appeared metabolically suppressed and failed to increase either aerobic glycolysis or mitochondrial oxidative metabolism upon stimulation. Conversely, B cells chronically exposed to high levels of BAFF were poised for rapid induction of aerobic glycolysis and metabolic reprogramming. These metabolic changes were essential for proliferation and Ab production, as pharmacologic inhibition of glycolysis or genetic deletion of Glut1 impaired B cells and suppressed Ab production following immunization. Thus, B cells share some common shifts in the balance of lactate production to oxygen consumption to orchestrate the metabolic transition from resting to activation. PI3K/Akt pathway plays a posttranslational and coordinating role in the regulation of mitochondrial pathways. However, cMyc combined with the PI3K/Akt pathway to reprogram glucose metabolism. In this setting, it may be that cMyc expression is important to orchestrate the metabolic transition from resting to activation.

A key feature of B cell activation through either the BCR or TLR4 is to induce cell growth, proliferation, and Ab production. Despite the distinct signaling mechanisms of BCR and TLR4, B cells underwent similar metabolic reprogramming. Catabolic metabolism, such as lipid oxidation, sharply decreased, and cell growth was favored. Aerobic glycolysis has been shown to provide cancer cells and activated T cells with biosynthetic intermediates and to play a central feature in the rapid growth of these cells (6, 11). Consistent with previous reports (9, 10), we found that B cells also increased glycolysis upon activation. However, in contrast to T cells, B cells started with higher rates of glycolysis, and activated B cells proportionally increased mitochondrial oxygen consumption to maintain a balanced glycolytic and oxidative metabolism. The basis for increased oxygen consumption and oxidative metabolism is likely through increased glucose and glutamine oxidation, as lipids are conserved for cell growth rather than consumed for energy production. Surprisingly, however, efficient mitochondrial electron transport was not essential for Ab production. The role of mitochondrial metabolism in B cell activation and function remains uncertain, but differences from T cells or macrophages suggest that alternate signaling pathways contribute to B cell metabolic reprogramming, or that B cells have intrinsic metabolic distinctions.

It is evident that B cell metabolic reprogramming depends in part on cMyc. In T cells, HIF1α was shown dispensable, whereas cMyc was required for upregulation of glycolytic genes, including Glut1, upon activation (5). B cells were similarly found to be independent of HIF1α but reliant on cMyc upregulation to increase glycolysis. Also, like T cells (5), cMyc did not regulate all mitochondrial metabolic pathways, as lipid oxidation decreased and pyruvate oxidation increased after activation regardless of cMyc expression. The PI3K/Akt pathway has been shown to promote glucose uptake and glycolysis in BCR-stimulated B cells (10). In IL-4–stimulated B cells, however, the PI3K/Akt pathway appeared dispensable (10), with STAT6 instead required. In LPS-stimulated B cells, it is likely that cMyc combined with the PI3K/Akt pathway to reprogram glucose metabolism. In this setting, it may be that cMyc leads to induction of gene expression and the PI3K/Akt pathway plays a posttranslational and coordinating role to orchestrate the metabolic transition from resting to activation. Whereas these pathways may control glucose metabolic pathways, the regulation of mitochondrial pathways remains uncertain.

We also show in this study that tolerance strongly influences B cell metabolism. Self-reactive B cells that become anergic were...
found metabolically suppressed to both BCR and TLR4 ligation. The complete inability of anergic B cells to metabolically reprogram in response to BCR stimulation was likely due in part to desensitized Ag receptor (39, 41). In particular, failure to activate the PI3K/Akt signaling pathway (42) may prevent upregulation of glycolytic metabolism. LPS was able to partially induce glycolysis and mitochondrial oxygen consumption of anergic B cells. Therefore, tolerance does not lead to wholesale inhibition of metabolic reprogramming. It remains unclear to what extent anergic B cells have intrinsic metabolic defects relative to desensitized Ag receptor or TLR signals that fail to properly upregulate metabolic regulatory pathways. In both settings, however, metabolic reprogramming is decoupled from receptor stimulation in anergic B cells.

In contrast to B cell anergy, B cells chronically exposed to high levels of BAFF showed enhanced and more rapid metabolic reprogramming upon TLR4 ligation. In particular, aerobic glycolysis increased rapidly to transiently shift the balance of metabolism to predominantly glycolytic. Oxygen consumption also increased, albeit at a slower rate. The differential rates of glycolytic and mitochondrial metabolism in BAFF transgenic B cells may reflect altered activity of signaling pathways that control glycolysis relative to mitochondrial metabolism. Acute treatment with BAFF can activate the PI3K/Akt signaling pathway (29, 30), but we found this insufficient to alter B cell metabolism on its own. It may be that chronic exposure to BAFF or additional signals are necessary. Thus, failure to properly induce tolerance due to chronically high levels of BAFF primes B cells to rapidly reprogram to the glycolytic metabolism, essential for activated Ab-producing cells.

The broad upregulation of glycolytic and mitochondrial metabolism in B cell activation suggests that targeting these pathways may disrupt B cell Ab production. However, the balanced metabolism and use of multiple metabolic pathways may have allowed functional redundancy and provide B cells the ability to use a variety of metabolic fuels. We show in this study that 2-DG and DCA each suppress B cell proliferation and Ab production. 2-DG and DCA suppress the entire glucose metabolism pathway or direct pyruvate toward mitochondrial oxidation, respectively. Although 2-DG can also induce cell death through endoplasmic reticulum stress (43), inhibiting PDHK did not affect B cell activation or survival. DCA nevertheless suppressed glycolytic flux to reduce the ability of lactate dehydrogenase to recycle NAD+, thus potentially increasing reactive oxygen species production (44). Therefore, the equivalent inhibition of proliferation and Ab production by these two compounds suggests that maintenance of aerobic glycolysis and lactate production are key events in Ab production. It may also be that glucose-derived metabolites are essential for biosynthesis or signaling to support B cell activation, such as appears to be the case for glyceraldehyde 3-phosphate in T cell production of IFN-γ (15).
upon activation, showing complex regulation and expression of multiple glucose transporters. B cell expression of other glucose transporters has not been well established, but Glut1, Glut3, Glut4, Glut8, and Glut11 may all be expressed and contribute to B cell nutrient uptake (45). Our data show, however, that Glut1 plays an essential role among these glucose transporters to support B cells. These data also demonstrate an in vivo cell-intrinsic requirement for glucose uptake in normal B cell homeostasis and Ab production following immunization.

Overall, the role of lymphocyte metabolism in self-tolerance and autoimmunity is poorly understood. T cells in SLE patients have dysfunctional and increased metabolic activity, with hyperpolarized autoimmunity is poorly understood. T cells in SLE patients have a tolerant phenotype in autoimmune and inflammatory diseases. This provides new directions to suppress Ab production and promote a tolerant phenotype in autoimmune and inflammatory diseases.

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We acknowledge members of the Rathmell laboratory for assistance and of mitochondrial metabolism to prevent the glycolytic shift observed in T cells. Tolerance status impact both glycolysis and mitochondrial pathways to either suppress or poise B cells for metabolic reprogramming. The specific mechanistic basis for metabolic modulation in tolerance and autoimmunity remains uncertain. However, targeting glycolytic pathways to mimic the suppressed metabolic state of anergy was sufficient both in vitro and in vivo to disrupt Ab production. In addition, it is likely that specific B cell subsets may have metabolic distinctions that could be exploited to target distinct cell populations. Further understanding of the regulation of lymphocyte metabolic pathways may provide new directions to suppress Ab production and promote a tolerant phenotype in autoimmune and inflammatory diseases.

Disclosures
The authors have no financial conflicts of interest.

References